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Directional Variations of Mechanical Parameters in Rat Skin Depending on Maturation and Age

H. GERHARD VOGEL, M.D.

Hoechst Aktiengesellschaft, Frankfurt, F.R.G.

Mechanical properties of rat back skin at low loads and at failure were studied in 2 directions, e.g., perpendicular and longitudinal to body axis beginning with early maturation (from 1 week onwards) until senescence (at 24 mo). Anisotropic behavior, known for human skin, has also been found in rats. Surprisingly, the changes due to maturation and aging were not the same for one area of skin regardless of the direction. Ultimate extension was more influenced by the aging process in samples perpendicular to the body axis than in those parallel to body axis. Elongation at zero load, that means load not measurable under the described conditions, was higher in the longitudinal samples than in the perpendicular ones in young and very old animals, whereas this difference was absent in mature animals. In contrast, ultimate load, tensile strength and modulus of elasticity were higher in perpendicular samples than in samples longitudinal to the body axis for young and very old, but not for mature animals. Elongation at low loads or low stresses shows a different pattern than at medium loads or medium stresses when both directions are compared. Apparently, elements contributing to the mechanical properties in the various directions are differently influenced by the maturation and aging processes. Moreover, the elements contributing to the changes at low loads react differently to the aging process from those responsible for the effects at medium and high loads.

The basic facts underlying the obvious changes between the soft skin in young individuals, the strong skin in adults, and the flaccid skin in elderly people are still unknown to a large extent One example is the anisotropy of skin.

Directional differences in structure and function of human skin have been known since the early observations of Langer in 1861 [1]. Biomechanical properties of human skin show directional variations related to Langer's lines [2-13]. The anisotropic behavior of human skin has been demonstrated by several methods applying either uni-axial strain in various directions [4,5,7-15] or torsional loads [3,5,11,16,17]. Directional differences of mechanical properties have not only been shown in human skin but also in the skin of animals, such as rats [18], mice [19], rabbits [20,21], cats [22] and guinea pigs [23]. These differences may be related to the step-phenomenon [18,24] which has been found in rats, guinea pigs, rabbits, and dogs.

Since mechanical parameters of skin have been shown to be dependent on maturation and aging both in man [25-35] and animals [24,36-50] it seemed to be worthwhile to study the age dependence of mechanical anisotropy in rat skin.

MATERIALS AND METHODS

Male Sprague Dawley rats (Hannover strain, Zentralinstitut Hannover) were used in all studies. Young animals (1, 2, and 3 weeks) were delivered with their mothers from the breeder. The old rats (1 and 2 years) were kept on Altromin-diet in air-conditioned rooms until sacrifice. Each group was randomly divided into 2 blocks which were assigned as "perpendicular to body axis" or "longitudinal to body axis". Skin samples were obtained as described previously [18,24,39, 41,43,44,46,47,49,51,52]. After sacrifice in chloroform anesthesia the back skin was shaved. A flap of 5 × 5 cm size was removed and the *skin thickness* was measured by calipers. From each rat 2 dumbbell-shaped samples of 50 mm length and 4 mm width were punched out either longitudinal or perpendicular to the body axis allowing a gauge length between the clamps of 30 mm. Due to this size the comparison longitudinal versus perpendicular was only possible between animals and not within the same individual. In a total number of 40 animals per age group, 2 samples per rat were punched out perpendicular to the body axis, in another 40 animals per age group the direction was longitudinal to the body axis. Thus, for each age group 80 samples obtained perpendicular to the body axis were compared with 80 samples obtained longitudinal to the body axis. Stress strain curves were measured at a strain rate of 50 mm/min with an Instron-instrument; whereby the 1st part of the curve was registered with 10-fold amplification. Even with this high amplification the stress-strain curve did not rise immediately. The force at this part at this amplification was below 0.005 N and therefore considered as zero load. In spite of great variations due to the conditions of clamping this horizontal part could be measured as *elongation at zero load*. At the point of failure the following parameters could be measured and calculated: *ultimate extension*, *ultimate load* and *tensile strength* (= ultimate stress = ultimate load divided by cross section area). From the straight part at the upper end of the stress-strain curve the *modulus of elasticity* was calculated (Fig 1). Furthermore, for each curve the extension was measured at given load interval

(0.05; 0.1; 0.2; 0.5; 1; 2; 5; 10; 20; and 50 N)

and at given stresses

(0.01; 0.02; 0.05; 0.1; 0.5; 1; 2; 5 and 10 N/cm^2).

Each parameter was measured longitudinal and perpendicular to the body axis and compared within each age group. Furthermore, for each direction the values were compared with the maximum and minimum value. Standard deviations for each value are shown in the following figures.

RESULTS

As expected, *body weight* and *skin thickness* of both groups perpendicular and longitudinal to body axis were identical at each age interval, indicating complete randomization within each age group (Fig 2). In this and the following figures age is plotted on the *abscissa* in a logarithmic scale. *Body weight* reaches the maximum at 12 mo and decreases thereafter as reported previously [24,44,46,49]. *Skin thickness* shows a similar pattern as body weight during late maturation (Fig 2) but not in very young animals. Additional to the findings of earlier studies [39,41,45] in this experimental series also the behavior during very early maturation (1, 2 and 4 weeks) was studied. Skin thickness decreases significantly between 1 and 3 weeks of age. After the minimum at 3 weeks the usual increase up to the maximum of 12 mo was seen. The decrease in skin thickness between 1 and 3 weeks may be explained due to water loss.

Fig 3 shows the age-dependence of *ultimate extension.* Remarkable differences between longitudinal and perpendicular samples are noted. Samples obtained perpendicular to the body axis showed an increase during maturation, reaching a maximum at 4 mo, and a decrease during aging as reported previously [45,49]. The behavior of samples obtained longitudinal to the body axis was quite different. After an initial rise a maxi-

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Reprint requests to: Prof. Dr. H.G. Vogel, Hoechst Aktiengesellschaft, Pharma Forschung, D-6230 Frankfurt/M. 80, F.R.G.

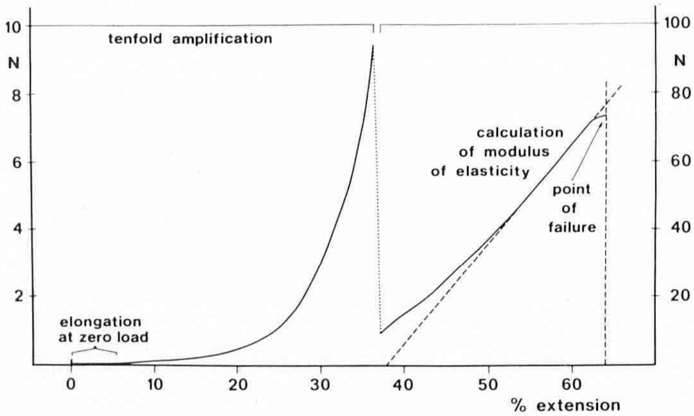

FIG 1. Original stress-strain curve.

mum was found at 3 weeks. Afterwards a slight decrease was noted until a minimum was reached at 12 mo. The value of ultimate extension in the 24-mo-old rats again was higher than at 12 mo. Generally, the changes due to maturation and age were much less in samples longitudinal than in those perpendicular to the body axis. Between 1 and 4 weeks all values of ultimate extension were significantly higher in samples punched out longitudinal to the body axis; between 4 mo and 12 mo they were considerably lower ($p < 0.001$); and at 24 mo they were virtually the same as in the samples obtained perpendicular to body axis.

Some of the differences found at ultimate extension are already reflected in *elongation at zero load* (Fig 4). In young animals (1 week to 1 mo) elongation at zero load was significantly higher ($p < 0.001$) in samples obtained longitudinal to body axis than in perpendicular samples. These differences disappeared between 2 and 12 mo. At 24 mo, however, the difference in the same direction was again significant ($p < 0.001$).

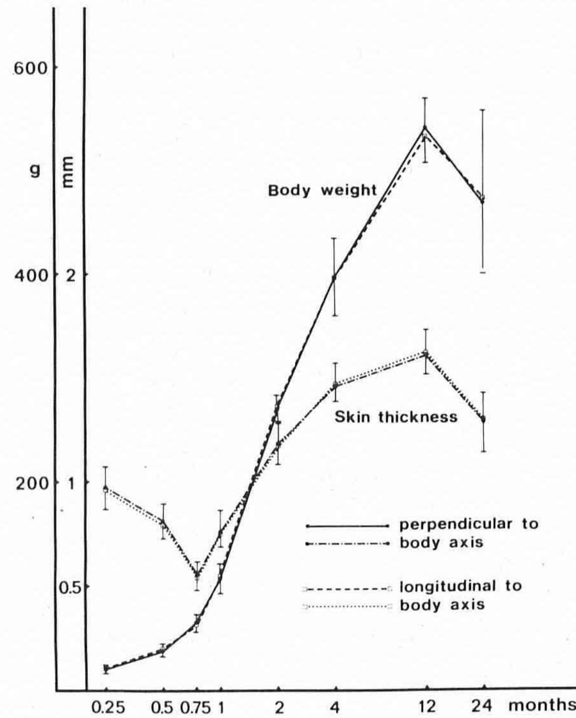

FIG 2. Body weight and skin thickness.

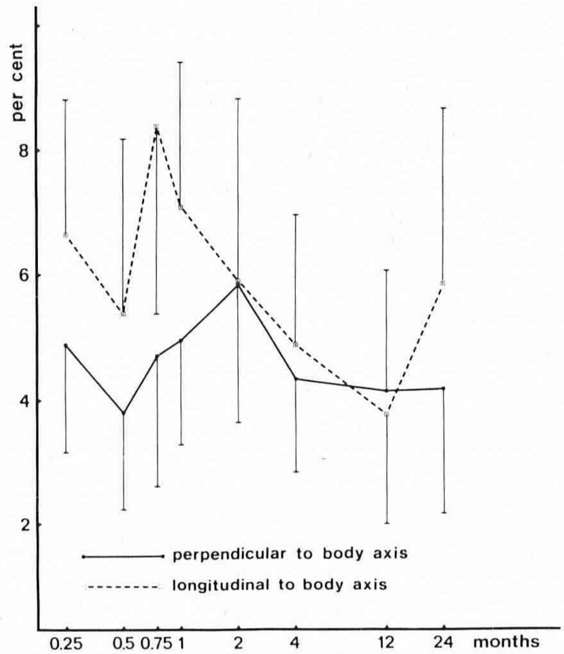

FIG 4. Elongation at zero load.

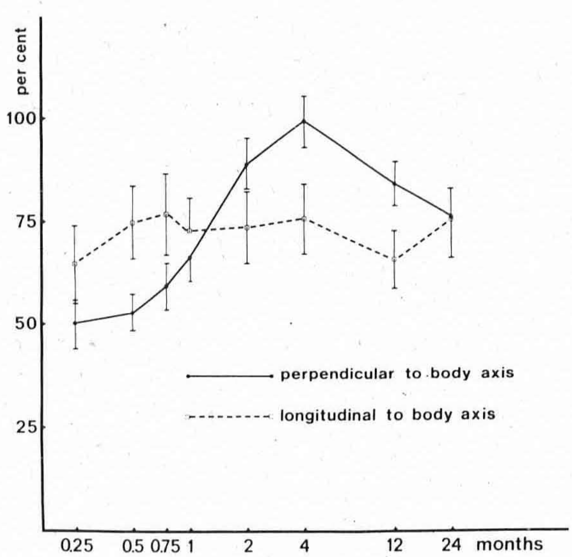

FIG 3. Ultimate extension.

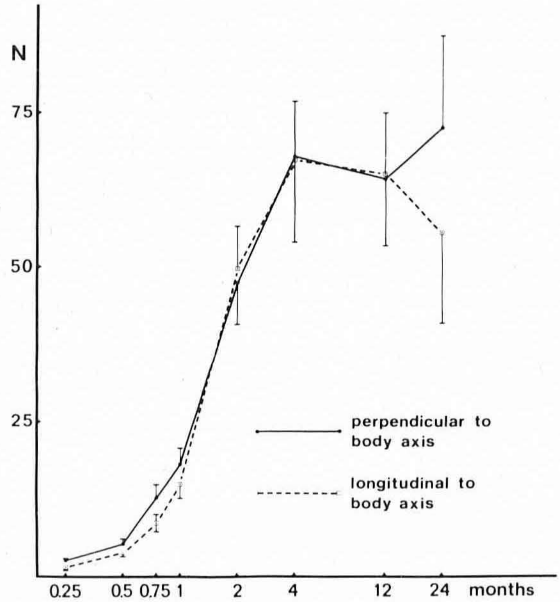

FIG 5. Ultimate load.

In young animals (up to 1 mo) and also in old animals (24 mo) *ultimate load* is significantly lower ($p <0.001$) for samples obtained longitudinal to body axis than for perpendicular samples (Fig 5). Between 2 and 12 mo no difference is found. Only the perpendicular samples show an unequivocal maximum at 4 mo.

Surprisingly, the decrease between 12 and 24 mo to be attributed to senescence was found for *ultimate extension perpendicular* to body axis and for *ultimate load longitudinal* to body axis.

Due to identical skin thickness of both groups *tensile strength* (Fig 6) shows a similar pattern as ultimate load. The step observed in both curves between 3 and 4 weeks is due to the minimum of skin thickness at 3 weeks. From 1 to 4 weeks tensile strength values are significantly lower ($p <0.001$) for longitudinal samples as they are in 24-mo old rats. Between 2 and 12 mo values are virtually identical.

Ultimate modulus of elasticity likewise shows lower values in longitudinal samples for 1- to 4-week-old-animals and for aged rats (Fig 7). Again, a step between 3 and 4 weeks was noted in samples perpendicular to body axis. Between 2 and 12 mo, however, the values longitudinal to body axis are higher than the values of samples punched out perpendicular to the body axis. However, this difference reaches only the value of $p <0.01$ whereas all other differences called significant exceed $p <0.001$. As with ultimate load only for samples longitudinal to body axis the maximum after maturation at 4 mo and the unequivocal decrease due to senescence was found.

Another approach to evaluate the mechanical behavior of skin is to measure the extension depending on logarithm of load or stress starting from very low values.

Fig 8*a* presents the elongation depending on the logarithm of stress values. This logarithmic transformation was chosen in order to elucidate especially the changes at low stresses. Age as logarithm of time is depicted on the depth axis. At 1, 2, 3 and 4 weeks of age extension is higher in all samples longitudinal to body axis compared to perpendicular samples at all stress values up to rupture. At an age of 2, 4 and 12 mo an interesting phenomenon is observed. At low stresses (0.01 to 0.2 N/mm^2) still longitudinal samples show the higher extension whereas at stress values from 0.5 N/mm^2 upwards the perpendicular samples show the higher extension. In these age groups the crossing

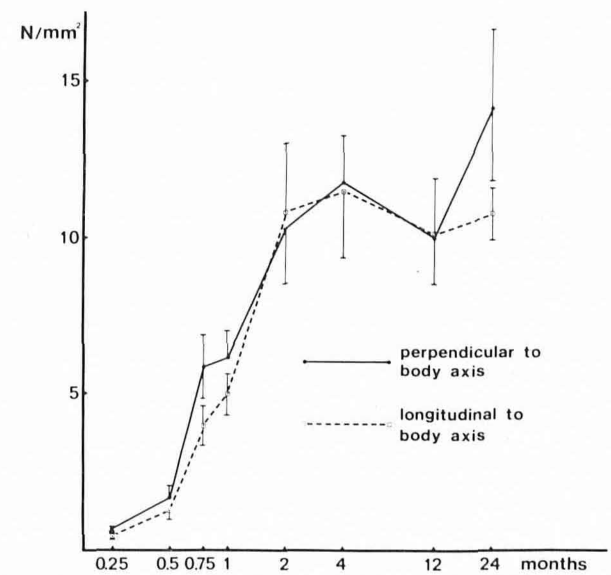

FIG 6. Tensile strength.

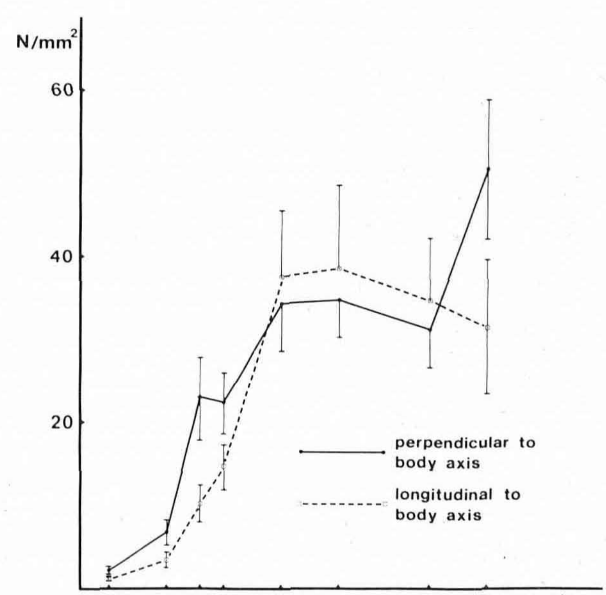

FIG 7. Ultimate modulus of elasticity.

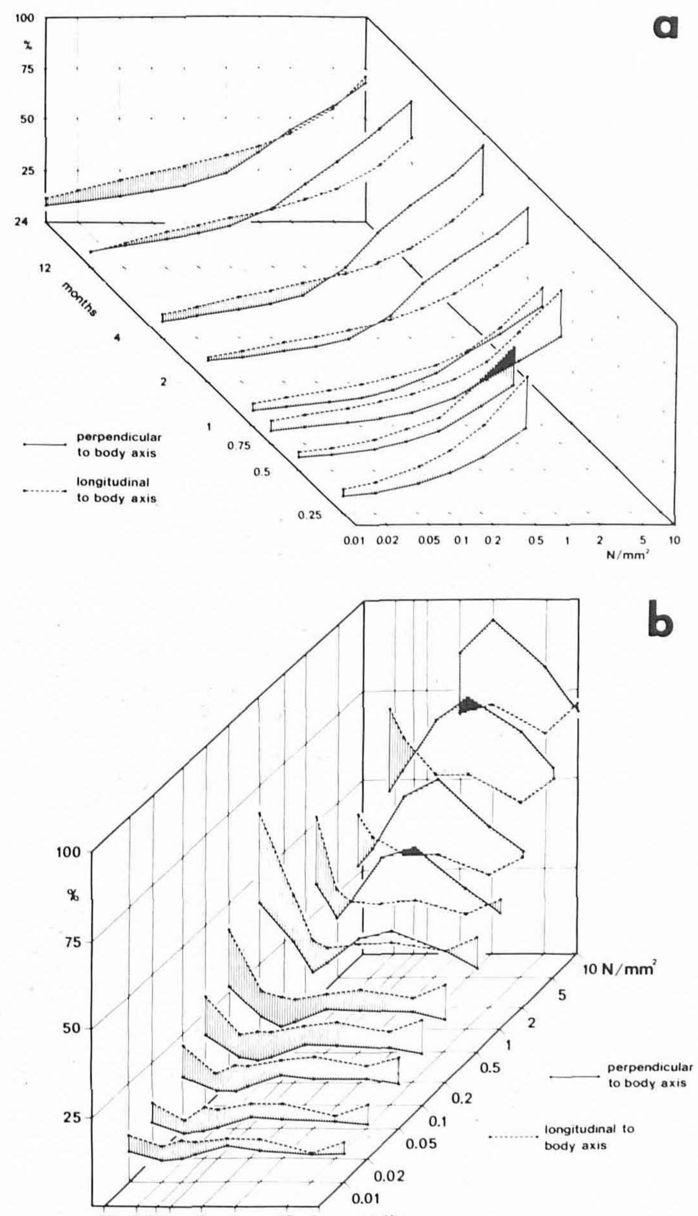

FIG 8. *a* and *b*, Elongation at various stress values.

of the stress-strain curves occurred at load values between 2 N and 5 N or at stress values between 0.2 and 0.5 N/mm^2.

Another phenomenon becomes more evident, when logarithm of age is chosen as *abscissa* and logarithm of stress values as *depth axis* (Fig 8*b*). At low stresses elongation decreases from 1 to 2 or 3 weeks and rises afterwards. This behavior is more prominent in perpendicular samples. At higher stresses and at an age beyond 2 mo the crossing of the values is noted again. Under these conditions the elongation values of perpendicular samples are higher than in longitudinal samples. At an age of 24 mo these differences almost disappear.

DISCUSSION

The results reported here confirm earlier observations in rat skin [39,41,45,46,47,49,50], however, give additional aspects. Analysis of the low part of the stress-strain curves [47] revealed the step phenomenon [18,24] which is dependent on the direction versus body axis. If only one direction is tested for reasons of standardization the influence of anisotropy may be neglected. On the other hand anisotropy or directional variations of mechanical parameters are very well-known for human and animal skin [3,5,7,8,10–14,16–23,53,54].

One would expect that these differences are related to the basic structure of skin and changes due to maturation and aging are the same for one area of skin regardless to the direction. Surprisingly, these studies have shown that this is not true. In any physical model to be used one has to imagine some finite elements contributing to the mechanical properties. Apparently elements contributing to mechanical properties perpendicular to body axis are differently influenced by the maturation and aging processes from those responsible for mechanical parameters parallel to body axis. Moreover, elements responsible for mechanical properties at high and medium loads are differently influenced by the maturation and aging process from those attributing to mechanical properties at low loads.

Age-dependent changes of mechanical properties of connective tissue may be explained by different degrees of crosslinking of collagen [40,41] indicated by its degree of solubility. This, however, does not explain the data reported here. One has to assume that the predominant orientation of the fiber network and therefore also the orientation of crosslinks changes with maturation and age.

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Announcement

The Fourth Annual Westwood Carolina Conference on Clinical Dermatology will take place October 22–25, 1981 at the Hyatt Hotel at Palmetto Dunes, Hilton Head Island, South Carolina. For information and registration, contact Dermatology Educational Services, Post Office Box 4207, Kenmore, New York 14217 or call (716) 884-1758.